ELECTROCHEMICAL BIOMARKER FOR DNA OXIDATIVE LESIONS

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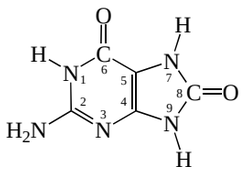
Electrochemical oxidation of DNA can occur at each of the four bases and guanine is the one that can suffer easier oxidative damage. The occurrence of the guanine oxidation product, 8-oxoguanine, as a consequence of DNA damage caused by DNA oxidation causes important mutagenic lesions and hence it is very important to develop reliable methods for its quantification.

The *in vivo* oxidation of chromosomic and mitochondrial DNA causes cell damage and plays an important, and probably the central, role in mutagenesis, carcinogenesis, and has been proposed to be a major contributor to ageing and other age-related diseases.

As a consequence of the oxidative lesion of DNA the compound commonly referred to as 8-oxoguanine (7,8-dihydro-8-oxoguanine or 8-oxoG) has been identified as the product of oxidation of guanine in the C8 position and the structure is



The formation of 8-oxoguanine in the DNA moiety, considered the most commonly measured product of DNA oxidation, causes important mutagenic lesions. In the DNA double helix this adduct pairs more easily with adenine (A) than with cytosine (C). This could lead to the substitution of cytosine in the complementary chain by adenine, which in turn leads to the substitution of the original guanine (G) by thymine (T). Through this consecutive series of events, an oxidative injury to DNA could result by mutagenic transversion of the type G → T. A mutation of G:C to T:A could then occur and be the starting point for a cellular dysfunction (malfunction), which in turn could lead to a state of illness. This transversion mutation is also often observed spontaneously in many tumour cells.

In cancer tissues, as well as in lung tissues of smokers, the levels of 8-oxoguanine found are higher than in healthy tissues, where a steady-state level exists due to the normal products of metabolism that oxidise DNA. Consequently this compound has been proposed as a urine biomarker for DNA oxidative lesions.

In this work an electrochemical study of the mechanism of oxidation of 8-oxoguanine on glassy carbon is presented.

The electrochemical oxidation of 8-oxoguanine is a reversible electrode process, Fig. 1. It is pH dependent and involves several reaction products in a multistep process in which a main peak is observed corresponding to the oxidation of 8-oxoguanine followed by other small peaks due to 8-oxoguanine oxidation products.

The results showed that 8-oxoguanine is more easily oxidised than guanine, which has a much higher oxidation potential for the same experimental conditions.

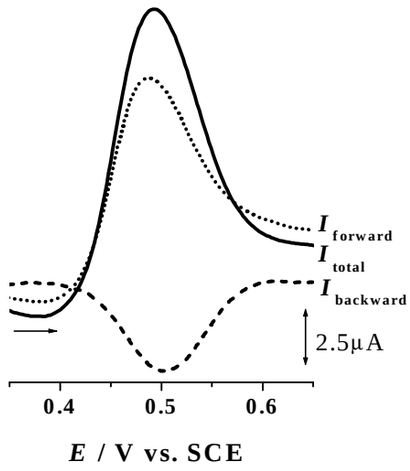


Fig. 1 Square wave voltammogram of 0.10 mM 8-oxoguanine in pH 4.5 0.1M acetate buffer. Pulse amplitude 50mV, step potential 2mV, frequency 50Hz.

This demonstrates that is possible to easily distinguish these two compounds, if both are present in a same sample, using differential pulse voltammetry, Fig. 2. Electroanalytical determinations of 8-oxoguanine can be done and the detection limit found was $8x10^{-7}$ M.

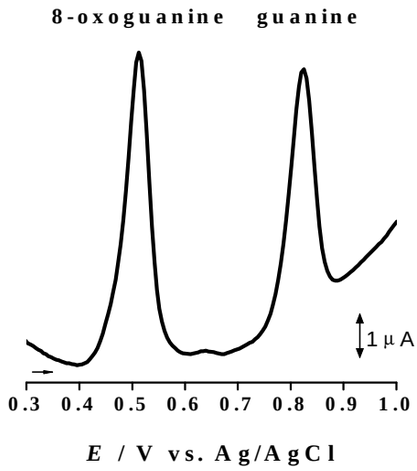


Fig. 2 – Differential pulse voltammogram of 0.10 mM 8-oxoguanine and 0.09 mM guanine in pH 4.5 0.1 M acetate buffer. Conditioning potential of +1.4 V during 180s before the scan. Pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}, electrode held at 0 V during 5 s between each potential scan.

Since 8-oxoguanine from the diet is not assimilated by the organism all the secreted 8-oxoguanine detected, usually in urine, is a direct consequence of DNA oxidation. This compound has been proposed as a urine biomarker for DNA oxidative lesions, establishing a key step in the effort to link oxidant formation to biomolecular damage and disease initiation and progression in a causative fashion.

This electroanalytical procedure can be applied to determinations of 8-oxoguanine in biological fluids such as serum or urine, of patients suffering from oxidative stress and which can be the cause of other disorders such as cancer or age related diseases.